AMENDMENTS

Amendments to the Claims

1-34 (Canceled).

35. (Currently amended) A method of determining protease activity of botulinum toxin serotype A (BoNT/A), comprising the steps of:

(a) treating a BoNT/A substrate with a sample under conditions suitable for clostridial toxin protease activity, said BoNT/A substrate comprising

(i) a donor fluorophore;

(ii) an acceptor having an absorbance spectrum overlapping the emission spectrum of said donor fluorophore; and

(iii) a BoNT/A recognition sequence comprising a BoNT/A P_5-P_4-P_3-P_2-P_1-P_1'-P_2'-P_3'-P_4'-P_5' cleavage site ~~region~~ sequence, said BoNT/A P_5-P_4-P_3-P_2-P_1-P_1'-P_2'-P_3'-P_4'-P_5' cleavage site ~~region~~ sequence intervening between said donor fluorophore and said acceptor;

wherein, under the appropriate conditions, resonance energy transfer is exhibited between said donor fluorophore and said acceptor;

(b) exciting said donor fluorophore; and

(c) determining resonance energy transfer of said treated substrate relative to a control substrate, wherein a difference in resonance energy transfer of said treated substrate as compared to said control substrate is indicative of BoNT/A protease activity.

36. (Currently amended) The method of claim 35, wherein said BoNT/A P_5-P_4-P_3-P_2-P_1-P_1'-P_2'-P_3'-P_4'-P_5' cleavage site ~~region~~ sequence comprises SEQ ID NO: 1.

37. (Presently presented) The method of claim 35, wherein said BoNT/A recognition sequence comprises the amino acid sequence selected from the group consisting of SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, amino acid residues 137 to 206 of SEQ ID NO: 2, amino acid residues 134 to 206 of SEQ ID NO: 2 and SEQ ID NO: 2.

38. (Original) The method of claim 35, wherein said sample is a crude cell lysate.

39. (Presently presented) The method of claim 35, wherein said sample is isolated clostridial toxin.

40. (Presently presented) The method of claim 35, wherein said sample is isolated clostridial toxin light chain.

41. (Original) The method of claim 35, wherein said sample is a formulated clostridial toxin product.

42. (Previously presented) The method of claim 35, wherein said sample is formulated BoNT/A product containing human serum albumin.

43. (Presently presented) The method of claim 35, wherein said acceptor is a fluorophore and step (c) comprises detecting donor fluorescence intensity of said treated substrate, wherein an increase in substrate cleavage results in an increase in donor fluorescence intensity of said treated substrate as compared to said control substrate, said increased donor fluorescence intensity being indicative of BoNT/A protease activity.

44. (Presently presented) The method of claim 35, wherein said acceptor is a fluorophore and step (c) comprises detecting acceptor fluorescence intensity of said treated substrate, wherein an increase in substrate cleavage results in a decrease in acceptor

fluorescence intensity of said treated substrate as compared to said control substrate, said decreased acceptor fluorescence intensity being indicative of BoNT/A protease activity.

45. (Presently presented) The method of claim 35, wherein said acceptor is a fluorophore and step (c) comprises detecting an acceptor emission maximum and a donor fluorophore emission maximum, wherein an increase in substrate cleavage results in a shift in emission maxima from near said acceptor emission maximum to near said donor fluorophore emission maximum, said shift in emission maxima being indicative of BoNT/A protease activity.

46. (Presently presented) The method of claim 35, wherein said acceptor is a fluorophore and step (c) comprises detecting the ratio of fluorescence amplitudes near an acceptor emission maximum over the fluorescence amplitudes near a donor fluorophore emission maximum, wherein an increase in substrate cleavage results in a decreased ratio of said treated substrate as compared to said control substrate, said decreased ratio being indicative of BoNT/A protease activity.

47. (Presently presented) The method of claim 35, wherein said acceptor is a fluorophore and step (c) comprises detecting the excited state lifetime of the donor fluorophore of said treated substrate, wherein an increase in substrate cleavage results in an increase in donor fluorophore excited state lifetime of said treated substrate as compared to said control substrate, said increased excited state lifetime being indicative of BoNT/A protease activity.

48. (Original) The method of claim 35, further comprising repeating step (c) at one or more later time intervals.

49. (Presently presented) The method of claim 35, wherein at least 90% of said BoNT/A substrate is cleaved.

50. (Presently presented) The method of claim 35, wherein at most 25% of said BoNT/A substrate is cleaved.

51. (Presently presented) The method of claim 50, wherein at most 15% of said BoNT/A substrate is cleaved.

52. (Presently presented) The method of claim 51, wherein at most 5% of said BoNT/A substrate is cleaved.

53. (Original) The method of claim 35, wherein the conditions suitable for clostridial toxin protease activity are selected such that the assay is linear.

54. (Presently presented) The method of claim 35, wherein said BoNT/A substrate comprises at most 20 residues.

55. (Presently presented) The method of claim 35, wherein said BoNT/A substrate comprises at most 40 residues.

56. (Presently presented) The method of claim 35, wherein said BoNT/A substrate comprises at most 50 residues.

57. (Presently presented) The method of claim 35, wherein said BoNT/A substrate comprises at most 100 residues.

58. (Presently presented) The method of claim 35, wherein said BoNT/A substrate comprises at most 150 residues.

59. (Presently presented) The method of claim 35, wherein said BoNT/A substrate comprises at most 200 residues.

60. (Currently amended) A method of determining protease activity of botulinum toxin serotype A (BoNT/A), comprising the steps of:

(a) treating a BoNT/A substrate with a sample under conditions suitable for clostridial toxin protease activity, said BoNT/A substrate comprising

(i) a donor fluorophore;

(ii) an acceptor having an absorbance spectrum overlapping the emission spectrum of said donor fluorophore; and

(iii) a BoNT/A recognition sequence comprising a BoNT/A P_5-P_4-P_3-P_2-P_1-P_1'-P_2'-P_3'-P_4'-P_5' cleavage site ~~region~~ sequence including a BoNT/A P_1-P_1' cleavage site, said BoNT/A P_1-P_1' cleavage site intervening between said donor fluorophore and said acceptor;

wherein either said donor fluorophore or said acceptor is not positioned within said BoNT/A P_5-P_4-P_3-P_2-P_1-P_1'-P_2'-P_3'-P_4'-P_5' cleavage site ~~region~~ sequence; and

wherein, under the appropriate conditions, resonance energy transfer is exhibited between said donor fluorophore and said acceptor;

(b) exciting said donor fluorophore; and

(c) determining resonance energy transfer of said treated substrate relative to a control substrate, wherein a difference in resonance energy transfer of said treated substrate as compared to said control substrate is indicative of BoNT/A protease activity.

61. (Currently amended) The method of claim 60, wherein said BoNT/A P_5-P_4-P_3-P_2-P_1-P_1'-P_2'-P_3'-P_4'-P_5' cleavage site ~~region~~ sequence comprises at least six consecutive residues of SEQ ID NO: 2, said six consecutive residues comprising Gln_{197}-Arg_{198}.

62. (Canceled).

63. (Previously presented) The method of claim 60, wherein said sample is a crude cell lysate.

64. (Presently presented) The method of claim 60, wherein said sample is isolated clostridial toxin.

65. (Presently presented) The method of claim 60, wherein said sample is isolated clostridial toxin light chain.

66. (Previously presented) The method of claim 60, wherein said sample is a formulated clostridial toxin product.

67. (Previously presented) The method of claim 60, wherein said sample is formulated BoNT/A product containing human serum albumin.

68. (Presently presented) The method of claim 60, wherein said acceptor is a fluorophore and step (c) comprises detecting donor fluorescence intensity of said treated substrate, wherein an increase in substrate cleavage results in an increase in donor fluorescence intensity of said treated substrate as compared to said control substrate, said increased donor fluorescence intensity being indicative of BoNT/A protease activity.

69. (Presently presented) The method of claim 60, wherein said acceptor is a fluorophore and step (c) comprises detecting acceptor fluorescence intensity of said treated substrate, wherein an increase in substrate cleavage results in a decrease in acceptor fluorescence intensity of said treated substrate as compared to said control substrate, said decreased acceptor fluorescence intensity being indicative of BoNT/A protease activity.

70. (Presently presented) The method of claim 60, wherein said acceptor is a fluorophore and step (c) comprises detecting an acceptor emission maximum and a donor fluorophore emission maximum, wherein an increase in substrate cleavage results in a shift in emission maxima from near said acceptor emission maximum to near said donor

fluorophore emission maximum, said shift in emission maxima being indicative of BoNT/A protease activity.

71. (Presently presented) The method of claim 60, wherein said acceptor is a fluorophore and step (c) comprises detecting the ratio of fluorescence amplitudes near an acceptor emission maximum over the fluorescence amplitudes near a donor fluorophore emission maximum, wherein an increase in substrate cleavage results in a decreased ratio of said treated substrate as compared to said control substrate, said decreased ratio being indicative of BoNT/A protease activity.

72. (Presently presented) The method of claim 60, wherein said acceptor is a fluorophore and step (c) comprises detecting the excited state lifetime of the donor fluorophore of said treated substrate, wherein an increase in substrate cleavage results in an increase in donor fluorophore excited state lifetime of said treated substrate as compared to said control substrate, said decreased ratio being indicative of BoNT/A protease activity.

73. (Previously presented) The method of claim 60, further comprising repeating step (c) at one or more later time intervals.

74. (Currently amended) The method of claim 60, wherein at least 90% of said BoNT/A ~~or BoNT/E~~ substrate is cleaved.

75. (Currently amended) The method of claim 60, wherein at most 25% of said BoNT/A ~~or BoNT/E~~ substrate is cleaved.

76. (Currently amended) The method of claim 75, wherein at most 15% of said BoNT/A ~~or BoNT/E~~ substrate is cleaved.

77. (Currently amended) The method of claim 76, wherein at most 5% of said BoNT/A ~~or BoNT/E~~ substrate is cleaved.

78. (Previously presented) The method of claim 60, wherein the conditions suitable for clostridial toxin protease activity are selected such that the assay is linear.

79. (Currently amended) A method of determining protease activity of botulinum toxin serotype A (BoNT/A), comprising the steps of:

(a) treating a BoNT/A substrate with a sample under conditions suitable for clostridial toxin protease activity, said BoNT/A substrate comprising

(i) a donor fluorophore;

(ii) an acceptor having an absorbance spectrum overlapping the emission spectrum of said donor fluorophore; and

(iii) a BoNT/A recognition sequence comprising a BoNT/A P_5-P_4-P_3-P_2-P_1-P_1'-P_2'-P_3'-P_4'-P_5' cleavage site ~~region~~ sequence, said BoNT/A P_5-P_4-P_3-P_2-P_1-P_1'-P_2'-P_3'-P_4'-P_5' cleavage site ~~region~~ sequence intervening between said donor fluorophore and said acceptor;

wherein either of said donor fluorophore, said acceptor, or both said donor fluorophore and said acceptor are genetically encoded; and

wherein, under the appropriate conditions, resonance energy transfer is exhibited between said donor fluorophore;

(b) exciting said donor fluorophore; and

(c) determining resonance energy transfer of said treated substrate relative to a control substrate, wherein a difference in resonance energy transfer of said treated substrate as compared to said control substrate is indicative of BoNT/A protease activity.

80. (Previously presented) The method of claim 79, wherein said donor fluorophore is genetically encoded.

81. (Previously presented) The method of claim 79, wherein said acceptor is genetically encoded.

82. (Presently presented) The method of claim 79, wherein said donor fluorophore and said acceptor are genetically encoded.

83. (Currently amended) The method of claim 79, wherein said BoNT/A P_5-P_4-P_3-P_2-P_1-P_1'-P_2'-P_3'-P_4'-P_5' cleavage site ~~region~~ sequence comprises SEQ ID NO: 1.

84. (Presently presented) The method of claim 79, wherein said BoNT/A recognition sequence comprises the amino acid sequence selected from the group consisting of SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, amino acid residues 137 to 206 of SEQ ID NO: 2, amino acid residues 134 to 206 of SEQ ID NO: 2 and SEQ ID NO: 2.

85. (Previously presented) The method of claim 79, wherein said sample is a crude cell lysate.

86. (Previously presented) The method of claim 79 or 83, wherein said sample is isolated clostridial toxin.

87. (Previously presented) The method of claim 79 or 83, wherein said sample is isolated clostridial toxin light chain.

88. (Previously presented) The method of claim 79, wherein said sample is a formulated clostridial toxin product.

89. (Previously presented) The method of claim 79, wherein said sample is formulated BoNT/A product containing human serum albumin.

90. (Presently presented) The method of claim 79, wherein said acceptor is a fluorophore and step (c) comprises detecting donor fluorescence intensity of said treated substrate, wherein an increase in substrate cleavage results in an increase in donor fluorescence intensity of said treated substrate as compared to said control substrate, said increased donor fluorescence intensity being indicative of BoNT/A protease activity.

91. (Presently presented) The method of claim 79, wherein said acceptor is a fluorophore and step (c) comprises detecting acceptor fluorescence intensity of said treated substrate, wherein an increase in substrate cleavage results in a decrease in acceptor fluorescence intensity of said treated substrate as compared to said control substrate, said decreased acceptor fluorescence intensity being indicative of BoNT/A protease activity.

92. (Presently presented) The method of claim 79, wherein said acceptor is a fluorophore and step (c) comprises detecting an acceptor emission maximum and a donor fluorophore emission maximum, wherein an increase in substrate cleavage results in a shift in emission maxima from near said acceptor emission maximum to near said donor fluorophore emission maximum, said shift in emission maxima being indicative of BoNT/A protease activity.

93. (Presently presented) The method of claim 79, wherein said acceptor is a fluorophore and step (c) comprises detecting the ratio of fluorescence amplitudes near an acceptor emission maximum over the fluorescence amplitudes near a donor fluorophore emission maximum, wherein an increase in substrate cleavage results in a decreased ratio of said treated substrate as compared to said control substrate, said decreased ratio being indicative of BoNT/A protease activity.

94. (Presently presented) The method of claim 79, wherein said acceptor is a fluorophore and step (c) comprises detecting the excited state lifetime of the donor fluorophore of said treated substrate, wherein an increase in substrate cleavage results in an increase in donor fluorophore excited state lifetime of said treated substrate as compared to said

control substrate, said increased excited state lifetime being indicative of BoNT/A protease activity.

95. (Previously presented) The method of claim 79, further comprising repeating step (c) at one or more later time intervals.

96. (Presently presented) The method of claim 79, wherein at least 90% of said BoNT/A substrate is cleaved.

97. (Presently presented) The method of claim 79, wherein at most 25% of said BoNT/A substrate is cleaved.

98. (Presently presented) The method of claim 97, wherein at most 15% of said BoNT/A substrate is cleaved.

99. (Presently presented) The method of claim 98, wherein at most 5% of said BoNT/A substrate is cleaved.

100. (Previously presented) The method of claim 79, wherein the conditions suitable for clostridial toxin protease activity are selected such that the assay is linear.

101. (Presently presented) The method of claim 35, wherein said acceptor is a fluorophore and step (c) comprises detecting the ratio of fluorescence amplitudes near an donor emission maximum over the fluorescence amplitudes near a acceptor fluorophore emission maximum, wherein an increase in substrate cleavage results in an increased ratio in said treated substrate as compared to the control substrate, said increased ratio being indicative of BoNT/A protease activity.

102. (Presently presented) The method of claim 35, wherein said acceptor is a quencher and step (c) comprises detecting donor fluorescence intensity of said contacted cell, wherein an increase in substrate cleavage results in an increase in donor fluorescence

intensity of said treated substrate as compared to said control substrate, said increased donor fluorescence intensity being indicative of BoNT/A protease activity.

103. (Presently presented) The method of claim 35, wherein said BoNT/A substrate is selected from the group consisting of SEQ ID NO: 85, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94 and SEQ ID NO: 95.

104 (Currently amended) The method of claim 60, wherein said donor fluorophore is not positioned within said BoNT/A P_5-P_4-P_3-P_2-P_1-P_1'-P_2'-P_3'-P_4'-P_5' cleavage site ~~region~~ sequence.

105 (Currently amended) The method of claim 60, wherein said acceptor is not positioned within said BoNT/A P_5-P_4-P_3-P_2-P_1-P_1'-P_2'-P_3'-P_4'-P_5' cleavage site ~~region~~ sequence.

106. (Presently presented) The method of claim 60, wherein said acceptor is a fluorophore and step (c) comprises detecting the ratio of fluorescence amplitudes near an donor emission maximum over the fluorescence amplitudes near a acceptor fluorophore emission maximum, wherein an increase in substrate cleavage results in an increased ratio in said treated substrate as compared to the control substrate, said increased ratio being indicative of BoNT/A protease activity.

107. (Presently presented) The method of claim 60, wherein said acceptor is a quencher and step (c) comprises detecting donor fluorescence intensity of said contacted cell, wherein an increase in substrate cleavage results in an increase in donor fluorescence intensity of said treated substrate as compared to said control substrate, said increased donor fluorescence intensity being indicative of BoNT/A protease activity.

108. (Presently presented) The method of claim 60, wherein said BoNT/A substrate comprises at most 20 residues.

109. (Presently presented) The method of claim 60, wherein said BoNT/A substrate comprises at most 40 residues.

110. (Presently presented) The method of claim 60, wherein said BoNT/A substrate comprises at most 50 residues.

111. (Presently presented) The method of claim 60, wherein said BoNT/A substrate comprises at most 100 residues.

112. (Presently presented) The method of claim 60, wherein said donor fluorophore and said acceptor are separated by at most 10 residues.

113. (Presently presented) The method of claim 60, wherein said donor fluorophore and said acceptor are separated by at most 20 residues.

114. (Presently presented) The method of claim 60, wherein said donor fluorophore and said acceptor are separated by at most 30 residues.

115. (Presently presented) The method of claim 79, wherein said acceptor is a fluorophore and step (c) comprises detecting the ratio of fluorescence amplitudes near an donor emission maximum over the fluorescence amplitudes near a acceptor fluorophore emission maximum, wherein an increase in substrate cleavage results in an increased ratio in said treated substrate as compared to the control substrate, said increased ratio being indicative of BoNT/A protease activity.

116. (Presently presented) The method of claim 79, wherein said acceptor is a quencher and step (c) comprises detecting donor fluorescence intensity of said contacted cell, wherein an increase in substrate cleavage results in an increase in donor fluorescence intensity of said treated substrate as compared to said control substrate, said increased donor fluorescence intensity being indicative of BoNT/A protease activity.

117. (Presently presented) The method of either claim 80 or 82, wherein said donor fluorophore is selected from the group consisting of blue fluorescent protein, cyan fluorescent protein, green fluorescent protein, yellow fluorescent protein and red fluorescent protein.

118. (Presently presented) The method of either claim 81 or 82, wherein said acceptor is a fluorophore.

119. (Presently presented) The method of claim 118, wherein said acceptor fluorophore is selected from the group consisting of blue fluorescent protein, cyan fluorescent protein, green fluorescent protein, yellow fluorescent protein and red fluorescent protein.

120. (Presently presented) The method of claim 79, wherein said BoNT/A substrate comprises at most 300 residues.

121. (Presently presented) The method of claim 79, wherein said BoNT/A substrate comprises at most 400 residues.

122. (Presently presented) The method of claim 79, wherein said donor fluorophore and said acceptor are separated by at most 20 residues.

123. (Presently presented) The method of claim 79, wherein said donor fluorophore and said acceptor are separated by at most 40 residues.